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Plasmid manufacture is the bottleneck of the genetic medicine revolution

The global demand for DNA, primarily in the form of plasmid DNA, has risen dramatically in recent years. An upward trend driven largely by the advent of cell and gene therapies, at least until the outbreak of the Covid-19 pandemic. These state-of-the-art therapies, which are transforming the treatment of devastating diseases, come in a variety of forms. But all of them rely at some point, in either their manufacture or mode of action, on the scalable production of DNA.

Initially, plasmid DNA was primarily used in academia or in the laboratory setting. Its role in the pharmaceutical industry originated in the production of therapeutic proteins, where plasmids are still used for the expression of foreign genes in bacteria or mammalian cells. Many providers were therefore specifically set up to support this demand. Now however, demand for DNA in terms of both scale and quality has been transformed by a cell and gene therapy industry that is experiencing rapid growth.

Hundreds of biopharmaceutical companies are utilising DNA for clinical development, and it is also used within multiple products now licensed for commercial distribution. The FDA has reported a surge of cell and gene therapy products entering early development, evidenced by an upswing in the number of investigational new drug (IND) applications. It anticipates that as of 2020 it will receive more than 200 INDs per year [1]. The global cell and gene therapy market is therefore also experiencing substantial growth. Having been valued at $1 billion in 2018, it is projected to have grown to over $14 billion by 2025 [2]. As the industry has shifted rapidly towards the commercial sphere, there has been a sudden and urgent requirement for high-capacity, high-quality DNA production.

Unfortunately, scaling up manufacturing capacity for plasmid DNA is non-trivial, and DNA production has become a bottleneck for the industry. Contract manufacturers able to offer GMP quality plasmid DNA have built up long waiting lists and substantial backlogs. The sector is running the risk that its inability to provide quality DNA within tight timelines could stall progress of R&D pipelines and damage the expectations of the market and of future patients. The onus is on DNA manufacturers to find a way to increase capacity without compromising on quality.

The Covid-19 outbreak has exacerbated the problem. This year, the biopharmaceutical industry has been working at unprecedented speed to develop safe and effective vaccines against the SARS-CoV-2 coronavirus. Approximately 200+ industry and academic groups are working on vaccine candidate programmes in parallel, in an attempt to address the unprecedented public health crisis. Despite none of these vaccines yet being approved for use, with time of the essence, manufacturing has begun ‘‘at risk’’ to enable rapid distribution upon regulatory approval. As a result, multiple programs are looking at producing vaccines on a larger scale than ever typically required, simultaneously.

A diversity of vaccine modalities are in development, in order to maximise the chance of developing at least one that can generate a safe and efficacious countermeasure to Covid-19. Two of these types, mRNA and DNA vaccines (nucleic acid vaccines), rely on plasmid DNA in their manufacture. Unlike traditional vaccines,

https://doi.org/10.1016/j.drudis.2020.09.040

which aim to elicit an immune response by introducing antigens via a weakened virus or protein, nucleic acid vaccines directly administer a part of the virus' genetic code. These sequences are translated once in the body to produce viral proteins that elicit an immune response – most commonly for SARS-CoV-2, the ''spike'' protein.

Nucleic acid vaccines offer multiple advantages over traditional vaccines, the biggest of which, in the face of a pandemic, is the speed of production. Taking preclinical development as an example, traditional vaccines take on average a year and a half to reach Phase I [3]. In comparison, we have seen Covid-19 mRNA vaccines enter the clinic just a few months after the genetic sequence of SARS-CoV-2 was announced. This rapid pace of development makes it possible that the first vaccine approved for Covid-19 could also be the first nucleic acid vaccine ever approved for use in humans. Moreover, the speed of development allows nucleic acid vaccines a flexibility not possible with traditional vaccines, giving them distinct advantages in fighting novel viral pathogens as they emerge.

Many players are involved in the Covid-19 nucleic acid vaccine space, including big pharma, such as Pfizer, GSK and Sanofi, innovative biotechs, such as Biontech, Moderna, Curevac, Inovio and Touchlight, and academic groups, including Imperial College London. Current frontrunners are Moderna [4] and BioNTech/Pfizer [5], both of which are developing mRNA vaccines and have reached Phase III and Phase IIb/III respectively.

With clinical development well underway, attention is now firmly on scaling up manufacturing capacity to deliver the billions of doses that will be needed should these vaccines obtain regulatory approval and authorisation. Manufacturing mRNA and DNA vaccines requires significant quantities of DNA, which is used directly as the product in a DNA vaccine, or as a starting template for an enzymatic reaction for mRNA production. As an example, delivering one billion doses of an mRNA vaccine may require production of in excess of 1 kg of DNA. Using current plasmid DNA manufacturing techniques this is going to be a substantial feat and will require global collaborations between biotech, pharma and contract development and manufacturing organisations (CDMOs). To put it into perspective, Aldevron, the market leader in plasmid DNA manufacturing, recently noted that over half of the world's plasmid DNA manufacturing capacity will be required for just one Covid-19 mRNA vaccine [6].

With the combined requirements of the nucleic acid vaccine and cell and gene therapy industries, DNA manufacture has reached a position where demand vastly outweighs available capacity. Plasmid DNA manufacture has become the bottleneck of the genetic medicine revolution and DNA manufacturers must respond quickly and effectively.

Significant investments are already being made around the world to scale up plasmid manufacturing capacity in attempt to alleviate some of the pressure at the bottleneck. Aldevron, which currently has the largest GMP plasmid facility in the world, expects to open a new 189,000 sq. ft manufacturing facility in spring 2021, adding to its existing space in Fargo, North Dakota [7]. Cobra Biologics is expanding its DNA vaccines, gene, and immunotherapy production with a €20m plant in Matfors, Sweden [8]. Meanwhile, VGXI recently announced that it has closed on the purchase of greenfield for a new, expanded manufacturing facility in Conroe, Texas [9].

However, given the time and cost-investment required to build this extra capacity, it is becoming increasingly clear that it is the fundamentals of plasmid DNA manufacture that render it incapable of enabling the future of genetic medicine. Plasmid DNA is produced using *E. coli* fermentation methods in large stainless steel bioreactors. The process is inherently slow and expensive, with limited capacity, and is prone to batch failure. This can have damaging effects on clinical progress, as was seen in 2018 when Editas Medicine had to delay filing the IND application for its LCA10 program to accommodate delays in third-party manufacturing of the product candidate [10]. The purification process is similarly expensive, slow, and difficult to scale. As long as the industry relies on this antiquated technology, DNA manufacture will remain a significant bottleneck. Additionally, as well as being limited by long lead times and high capital and labour costs, the presence of antibiotic resistance genes in the final product is an ongoing concern of regulators worldwide.

Given these significant challenges, which threaten to stymie the genetic medicine revolution, companies and institutions are looking for alternative DNA manufacturing technologies. In the UK, the Coalition for Epidemic Preparedness Innovations (CEPI) has an ongoing request for proposals aiming to address the challenges of rapidly scaling nucleic acid vaccine manufacture – ''COVID-19: Accelerating vaccine development and global manufacturing capacity to stop the pandemic''.

As an alternative to plasmid DNA production, Touchlight has developed synthetic DNA manufacturing using an *in vitro* dual enzyme process. The company's proprietary synthetic DNA vector, which is known as ''doggybone'' or dbDNA™, is classified by regulatory authorities as a chemical, rather than a biological product. The technology has reached a level of maturity where it is uniquely positioned to enable the rapid, global-scale DNA production that is so urgently required. GMP dbDNA is manufactured at two sites – one in London and one at Touchlight AAV in Spain. In 2021, Touchlight is planning to more than triple its manufacturing capacity in London with the addition of 10 production suites, enabling production of over 1 kg of dbDNA per month. The technology is already being utilised by multiple mRNA companies in the race to develop a vaccine for Covid-19 and beyond. Rather than trying to reduce pressure at the bottleneck of plasmid manufacture by building more capacity, using enzymatic DNA technology it may be possible to eradicate the bottleneck entirely.

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